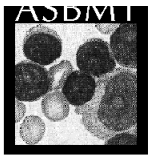

Pathophysiologic mechanisms of acute graft-vs.-host disease

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ABSTRACT

Graft-vs.-host disease (GVHD) remains the major toxicity of allogeneic bone marrow transplantation. Mechanistic studies in experimental animal models provide a better understanding of the complex relationships and cascade of events mediated by cellular and inflammatory factors. Also, advances in basic immunology have cleared the way for a more precise view of allogeneic reactions between donor and host. In addition, the use of mutant mice lacking critical cytolytic proteins has helped map out the molecular pathways by which GVHD targets organ damage. In this article, these mechanisms are reviewed and synthesized into a coherent conceptual framework, providing a state-of-the-art summary of the pathophysiology of acute GVHD.

KEY WORDS

Graft-vs.-host disease • Cytokine • Cytotoxicity • Antigen presentation

PATHOPHYSIOLOGY

Our understanding of the pathophysiology of graft-vs.-host disease (GVHD) has improved greatly with recent insights into the cellular and humoral interactions that are intrinsic to all inflammatory processes. In allogeneic bone marrow transplantation (BMT), donor lymphocytes are infused into a host that has been profoundly damaged. The effects of underlying disease, prior infection, and conditioning regimen may result in substantial proinflammatory changes in endothelial and epithelial cells. Donor cells rapidly encounter an environment that not only is foreign, but also has been altered to promote the activation and proliferation of inflammatory cells by the increased expression of adhesion molecules, cytokines, and cell surface recognition molecules. The donor lymphocytes respond in turn by reacting in a fashion that, under ordinary circumstances, would foster the control or resolution of infection. Thus, the pathophysiology of acute GVHD may be considered as a distortion of the cellular response to viral and gram-negative bacterial infection.

The principal target organs of GVHD also suggest a close relationship between infection and GVHD. The skin, gut, and liver all share an extensive exposure to endotoxins and other bacterial products that can trigger and amplify local inflammation. This exposure distinguishes them from organs like the heart and kidneys that are not GVHD targets. The lung is an organ of controversy in this regard. While the lungs are not classic GVHD targets, accumulating evidence suggests that they share some degree of GVHD susceptibility with the skin, gut, and liver [1,2]. Because of their situation as primary barriers to infection, these target organs have large populations of professional antigen-presenting cells (APCs), such as macrophages and dendritic cells, that may enhance the graft-vs.-host (GVH) reaction.

Recent findings implicate the excessive production of cytokines, the central regulatory molecules of the immune system, as well as cellular effectors in the induction and maintenance of experimental and clinical GVHD [3–5]. The pathophysiology of acute GVHD can be considered in a framework of three sequential phases [3,5].

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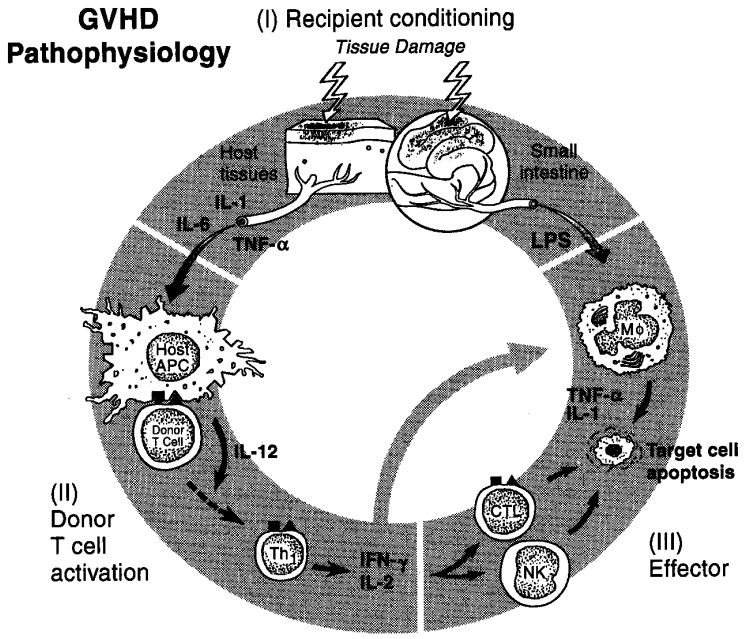

Figure 1. Acute GVHD pathophysiology

Pathologic mechanisms of GVHD as three sequential phases: I, recipient conditioning; II, donor T-cell activation, adhesion, costimulation, and cytokine production; III, inflammatory and cytolytic effectors.

PHASE 1

Conditioning r egimen

The earliest phase of acute GVHD starts before donor cells are infused (Fig. 1). The transplant conditioning regimen is an important variable in the pathogenesis of acute GVHD because it can damage and activate host tissues, including the intestinal mucosa, liver, and other tissues. Activated host cells secrete inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1 [6], and growth factors such as granulocyte-machrophage colony-stimulating factor (GM-CSF) [7–9]. The presence of inflammatory cytokines during this phase may upregulate adhesion molecules [10] and major histocompatibility complex (MHC) antigens [11–15], thereby enhancing the recognition of host MHC or minor histocompatibility antigens by mature donor T cells after the cellular component of the graft is infused. This scenario is in accordance with the observation that an enhanced risk of GVHD after clinical BMT is associated with intensive conditioning regimens that cause extensive injury to epithelial and endothelial surfaces with a subsequent release of inflammatory cytokines and increases in expression of cell surface adhesion molecules [16–18]. The relationship between conditioning intensity, inflammatory cytokines, and GVHD severity has been further supported in animal models [19]. Moreover, the risk of inducing severe acute GVHD appears to be less if the lymphocytes are infused well after the primary tissue injury has resolved [20,21].

PHASE 2

Donor T-cell activation

The second phase of acute GVHD includes antigen presentation, the activation of individual donor T cells, and the subsequent proliferation and differentiation of these activated T cells. When a $CD4^+$ cell enters the recipient blood stream, it interacts with the MHC class II molecules of the APCs, whereas a $CD8^+$ cell interacts with MHC class I antigens. Several lines of evidence suggest that host APCs are particularly important in GVH reactions, and eliminating host APCs of hematopoietic origin can promote tolerance and reduce GVHD [22,23]. When donor and recipient are not MHC identical, donor T cells can recognize host MHC molecules as foreign, and the resultant GVH reaction can be dramatic, even against single MHC antigen differences. In such cases, "nonprofessional APCs" that express MHC antigens may be targeted, depending on the expression of other costimulatory molecules (see below). When the recipient and donor are MHC identical, GVHD occurs through recognition by the T cell and its receptor (TCR) of different peptides, so-called minor histocompatibility (miH) antigens, bound to the same MHC. Because the manner of protein processing depends on genes outside of the MHC, two siblings will have many different peptides in the MHC groove. Therefore, one potential area to interfere with signal recognition is at the level of MHC-peptide-TCR interaction [24,25].

It is clear that GVHD after BMT from an MHC-identical sibling depends on the recognition of different allelic peptide products presented by the same (i.e., shared) MHC. The identification of these potential peptides has been an area of intense research. It remains unclear how many of these peptides behave as miH antigens, although the estimates from mouse studies suggest that 50 such antigens may exist. The precise number of these antigens in humans is not clearly defined. While there are many potential miH antigens, the actual numbers that can potentially induce GVHD ("major minors") are likely to be limited and will probably

prove less powerful stimulators of GVHD than MHC antigens. Recent clinical data suggest that mismatches of miH antigens between HLA-identical donors and recipients are associated with GVHD [26]. Of five previously characterized miH antigens (HA-1, -2, -3, -4, and -5) recognized by T cells in association with HLA-A1 and A2, mismatching of HA-1 alone was significantly correlated with acute grade II–IV GVHD ($p = 0.02$), and mismatching at HA-1, -2, -4, and -5 was also associated with GVHD ($p = 0.006$). In all cases where an HA-1–positive patient received an HA-1–negative graft, acute GVHD developed; a mismatch at HA-3 had no effect. Furthermore, peptide analysis of the HA-2 antigen suggests that it is a member of the class I myosin family [27].

The peptide itself can have profound consequences on the outcome of the interactions of the TCR-peptide-MHC. The recognition of slightly altered peptides can lead to partial activation of T cells [28–30]. Peptides with minor variations of sequence compared to native peptide may lead to significant changes in the T-cell response. Therefore the TCR should not be thought of as a simple on/off switch but rather as a rheostat, where subtle variations of peptide configuration might have helpful or deleterious effects on the ensuing immune response. Small changes in peptides may shift a vigorous proliferative response to the induction of an anergic state (or vice versa). These variations in peptides have been referred to as altered peptide ligands.

T-cell adhesion and costim ulation

When a mature T cell is suddenly placed into the circulation of an allogeneic host, it will travel through the bloodstream in a fashion similar to its journey in the donor. The systemic vasculature, including the capillary beds, represents the potential first and extensive area of contact with new alloantigens for this T cell. Vascular antigens have therefore been studied as potential miH antigens, and studies suggest that they may be important in the pathogenesis of GVHD, although the data are conflicting [31–33]. The degree to which donor T cells are stimulated by host vascular endothelium, which may act as a nonprofessional APC when activated, is currently under investigation.

Before antigen recognition and activation, a T cell must adhere long enough to a surface to become activated. The current view of this process is that a T cell rolls along the endothelial surfaces with its TCR in contact with a variety of different antigens. The on and off rates of binding of the TCR to the MHC molecules have been estimated, and the binding (K_m) of TCR to antigen is relatively weak, which raises the likelihood that the TCR will engage with a large number of MHC molecules [34]. If the TCR recognizes any particular antigen that results in activation of the T cell, adhesion molecules then firmly anchor the T cell and prevent any further rolling. Various integrins and selectins are critically important to this process [35]. Moreover, these anchors also provide the possibility of egress for T cells from the circulation into lymph nodes, spleen, reticuloendothelial tissues, and other target organs. Interference with adhesion could potentially weaken the interaction between the TCR and the MHC molecule, leading to a lower binding affinity and perhaps a weaker immune response. Alternatively, monoclonal antibodies

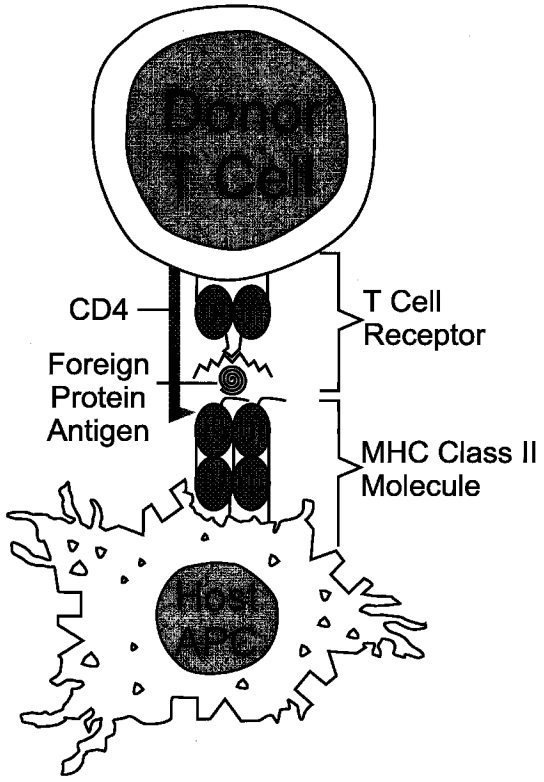

Figure 2. Donor T-cell interactions with host APC
Molecular diagram of a $CD4^+$ donor T cell recognizing foreign protein antigen via the T-cell receptor in the context of MHC II molecules.

that block the interaction of adhesion molecules with their ligands may physically prevent the dimerization of the TCR and MHC molecules and subsequent intracytoplasmic signaling. A polymer that binds to the MHC molecule or the peptide modeled after the D1-CD4 domain could also cause such interference.

T-cell activation requires two signals. The first signal is provided by the TCR-peptide-MHC interaction [36,37]. For donor T cells, this signal is the interaction between the allo-peptide bound to the host or donor MHC (Fig. 2). The second, or costimulatory signal, requires contact with APCs [38,39]. The second signal determines the outcome of the activation sequence, leading to complete activation, partial activation, or a long-lasting state of antigen-specific unresponsiveness, termed anergy. Several ligands can provide costimulation for resting T cells, antigen-primed T lymphocytes, and T-helper cell clones. The best-characterized costimulatory molecules are the B7 antigens, which bind to two T-cell surface receptors, CD28 and CTLA-4. In normal animals, the outcome of T-cell activation depends on a signal from the TCR, a costimulatory signal from CD28, and an inhibitory signal from CTLA-4. This process has been elegantly demonstrated in mice deficient for either CD28 or CTLA-4 [40,41]. Signaling through the TCR complex in the absence of costimulation results in a deactivation signal that leads to anergy. Conversely, absence of CTLA-4 results in loss of the inhibitory signal, resulting in enhanced and uncontrolled cytokine production and proliferation. The use

of CTLA-4 inhibition to induce donor T-cell anergy is currently being tested in a clinical phase I trial [42].

Costimulatory requirements for T cells depend on their state of activation-induced maturation. For resting (unprimed) $CD4^+$ T cells, VCAM-1 and intercellular activation molecule-1 (ICAM-1) (and possibly other molecules) have been demonstrated to provide the costimulatory signal or signals. Consequently, T-cell activation *in vivo* is very complex and depends on the state of activation of the T cell (resting vs. activated, naive vs. mature) as well as the nature of the APC (professional vs. nonprofessional, resting vs. activated).

Interactions of CD40 and its ligand (CD40L) are also important costimulatory signals for T-cell activation. Human endothelial cells express CD40, and the interaction with CD40L on the T cell can induce endothelial cell activation [38,43,44]. This activation can lead to increased expression of ICAM-1 (or CD54), E-selection (CD62E), and VCAM-1 on endothelial cells. These interactions suggest a mechanism whereby activated $CD4^+$ T cells may increase their own response by causing increased expression of endothelial cell surface adhesion molecules.

T-cell cytokines

T cells that secrete IL-2 and interferon (IFN)-γ (type 1 cytokines) are critical mediators of acute GVHD. The importance of GVHD has been demonstrated both experimentally and clinically. First, IL-2 is secreted by donor $CD4^+$ T cells in the first days after experimental allogeneic BMT [45]. Second, the blockade of IL-2 with antibodies to IL-2 or its receptor can inhibit the development of experimental disease [45]. Clinically, the precursor frequency of host-specific, IL-2–producing T cells (precursor frequency of helper T cells) is predictive for the risk of acute GVHD [46,47]. In addition, soluble IL-2 receptor levels may be a sensitive indicator of impending GVHD onset, and they correlate with disease severity [48].

Increased serum levels of IFN-γ are associated with acute GVHD, and lymphocytes from animals with GVHD secrete significantly greater amounts of IFN-γ than lymphocytes from controls without GVHD [49–53]. Additional evidence of a role for IFN-γ in experimental acute GVHD includes priming of macrophages by IFN-γ during acute GVHD to produce inflammatory cytokines [54], induction of pathology in skin tissues and the gastrointestinal tract by IFN-γ [55,56], suppression of T-lymphocyte function characteristic of acute GVHD by IFN-γ [57,58], prevention of acute GVHD when $CD8^+$ cells are incapable of IFN-γ production [59], and inhibition of acute GVHD by direct or indirect blockade of IFN-γ [55,60–62].

The preincubation of donor T cells in the presence of the Th2 cytokine, IL-4, can polarize these T cells toward a Th2 cytokine phenotype [61]. Transplantation of polarized Th2 T-cell populations failed to induce acute GVHD to MHC class I or class II antigens. These experiments strongly support the concept that the balance in Th1 and Th2 cytokines is critical for the development (or prevention) of acute GVHD. Further data show that Th2 cells maintain some antileukemic efficacy and can support lymphohematopoietic engraftment [62,63]. Data from experimental BMT systems suggest that the use of G-CSF to mobilize peripheral blood hematopoietic cells can lead to Th1→Th2 polarization of T cells in the stem cell innoculum, albeit indirectly, resulting in less GVHD than in saline-treated controls [64–66]. This effect also changes the production of other inflammatory cytokines such as TNF-α [67,68].

Regulatory cells may also help determine the ultimate response of donor T cells to host antigens. Double-negative T cells (usually $NK1.1^+$) can suppress a T-cell response in a mixed lymphocyte reaction (MLR) and can prevent GVHD *in vivo* [69]. Presumably, these regulatory cells develop to control the intensity of the overall response to a specific antigen. The balance between reactive T cells and suppressor T cells could thus control the intensity of GVHD. The generation and maintenance of these suppressor cells are poorly understood, although some effects may be related to differences in their cytokine milieu. Other potential avenues for tolerance induction may occur at the cellular level. Groux *et al.* [70] demonstrated that $CD4^+$ T cells, grown ex vivo in the prolonged presence of IL-10, suppressed inflammatory bowel disease that was induced by pathogenic T cells. These cells were termed "Tr1." Moreover, Tr1 cells have been isolated from the peripheral blood of severe combined immunodeficient patients after allogeneic stem cell transplantation, in which high levels of IL-10 *in vivo* are associated with donor/host tolerance [71]. These results suggest that prolonged exposure of naive $CD4^+$ T cells to IL-10 may result in a population of Tr1 cells that can regulate immune responses and modulate GVHD.

PHASE 3

Inflammatory effectors

The third phase of acute GVHD is complex and has only recently been appreciated. The initial hypothesis that the cytolytic function of cytotoxic T lymphocytes (CTLs) directly causes the majority of tissue damage in GVHD targets is too limited [72]. Large granular lymphocytes (LGLs) or natural killer (NK) cells appear to be prominent in the effector arm of GVHD in several animal models, and they may contribute to the pathologic damage, i.e., induce the changes of GVHD following the T-cell–mediated GVH reaction [72,73]. LGLs do not recognize HLA proteins as targets, but they can be recruited by cytokines released by T cells.

Mononuclear phagocytes, which have been primed with Th1 cytokines during phase 2, have an important role in this phase of acute GVHD. Monocytes receive a second, triggering signal to secrete the inflammatory cytokines TNF-α and IL-1. This stimulus may be provided by lipopolysaccharide (LPS) (endotoxin), which can leak through the intestinal mucosa damaged by the conditioning regimen and subsequently stimulate gut-associated lymphocytes and macrophages [54]. LPS reaching skin tissues may also stimulate keratinocytes, dermal fibroblasts, and macrophages to produce similar cytokines in the dermis and epidermis [7–9]. Recent experimental data suggest that TNFL production by donor cells in response to LPS is an important risk factor for GVHD severity independent of T-cell responses to host antigens [74]. TNF-α can cause direct tissue damage by inducing necrosis of target cells, or it may induce tissue destruction during GVHD through apoptosis (programmed cell death). The induction of apoptosis commonly occurs after activation of the TNF-α–Fas antigen pathway [75].

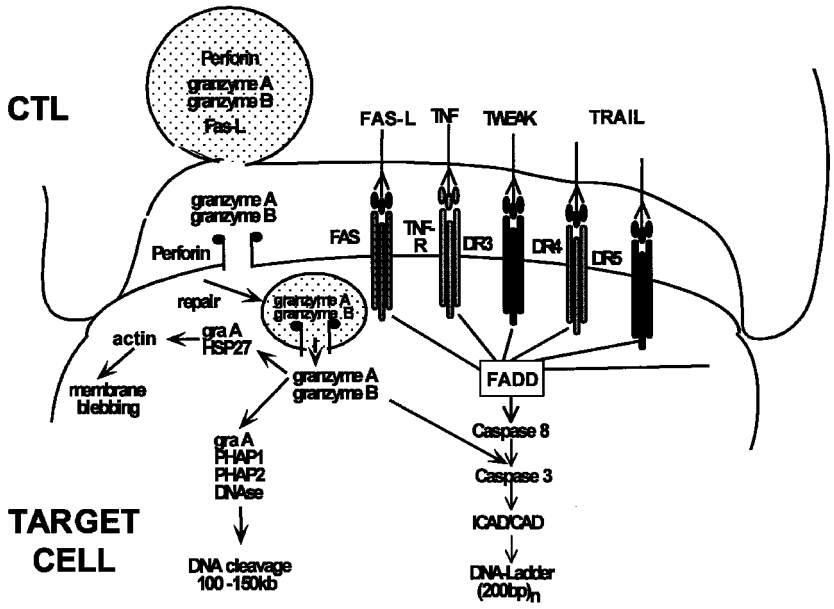

Figure 3. CTL effector pathways

The various components of the molecular pathways of CTL-mediated lysis. Fas, CD95/AP01; DR3, AP03/WSL1/TRAMP/LARD; DR4, TRAIL R1; DR5, TRAIL R2/TRICK 2, KILLER-DR5; TRAIL, AP02L.

Apoptosis is probably critical to GVHD in the large intestine [76] and skin [77,78] and possibly in endothelial cells [79]. In addition to these proinflammatory cytokines, excess nitric oxide (NO) produced by activated macrophages may contribute to the deleterious effects on GVHD target tissues, particularly immunosuppression [58,80,81]. Thus, the induction of inflammatory cytokines may synergize with the cellular damage caused by CTLs and NK cells [72,82], resulting in the amplification of local tissue injury and further promotion of an inflammatory response, which ultimately lead to the observed target tissue destruction in the BMT host.

The role of inflammatory cytokines in GVHD explains a number of unique and seemingly unrelated aspects of GVHD. For example, a number of analyses of clinical transplants noted increased risks of GVHD associated with advanced-stage leukemia, certain intensive conditioning regimens, and viral infections [16–18]. Similarly, the reduction in GVHD seen in gnotobiotic mice [83,84] and patients with aplastic anemia undergoing transplantation in laminar airflow environments with gut decontamination [85] may be explained by the reduction of bacterial LPS on the skin and gut. LPS may leak through damaged intestinal mucosal surfaces and stimulate the numerous gut-associated lymphocytes and macrophages to produce inflammatory cytokines. The beneficial effect of protective environments may be less apparent in patients receiving transplants for malignancies, because prior therapy and associated infections may have resulted in an environment that facilitates GVHD. Viral infections are also commonly associated with GVHD. They are more frequent in patients with GVHD, and a viral illness may cause the initiation of GVHD or worsening of established GVHD. Cytomegalovirus has a particularly close relationship with GVHD [86–89], as does herpes simplex virus [90–92], and possibly human herpes virus-6 [93]. The precise pathophysiology of this connection remains uncertain. While it has been hypothesized that viral antigen expression on target cells might function as a minor histocompatibility antigen, direct evidence proving this association is lacking. Certainly, cellular damage to the intestine or liver may increase the permeability of those organs, resulting in increased absorption of bacterial products such as LPS. Alternatively, GVHD targets could be innocent bystanders of either a virus-induced activated T-cell or an NK-cell attack [94,95].

Cytolytic effectors

Although cytokines clearly play important roles in the morbidity and mortality of systemic GVHD, they may be less important as mediators of damage in individual GVHD target organs. The unusual cluster of GVHD target organs (skin, gut, and liver) is not adequately explained by the systemic release of cytokines. For example, intravenous infusion of TNF-α and IL-1 does not cause the lymphomononuclear cell infiltration of liver and skin observed in GVHD. Furthermore, the absence of GVHD toxicity in other visceral organs, such as the kidneys, argues against circulating cytokines as the sole causation of tissue-specific damage.

Since cytotoxic T cells possess the capacity to kill virtually all nucleated cells, cell-mediated cytotoxicity is thought to contribute to the destruction of GVHD target tissues. T cells can effect cytolysis by either direct contact or the release of soluble mediators such as TNF-α. Contact-dependent cell-mediated cytotoxicity can occur through a secretory pathway involving granule release or by effector cell membrane ligand interaction with death receptors on the membrane of the target cell [96,97] (Fig. 3). After secretion of granules by the effector cell, the polymerization of perforin on binding to the target membrane is crucial to optimize penetration of granule contents,

including granzymes A and B, into the targeted cells. However, evidence suggests that perforin and granzymes may also gain access to the target cell cytosol by other means [98–100]. Apoptosis of target cells is then rapidly induced by granzyme B activation of the caspase cascade. This cascade ultimately results in the release of an inhibitor (ICAD) bound to a caspase-associated DNase molecule (CAD), which is followed by fragmentation of target cell DNA. A common pathway appears to operate in signaling through the Fas-associated death domain (FADD) of the so-called death receptors (DR). A number of ligands have been identified on T cells that possess the capability to trimerize TNFR-like DR molecules. In addition to the well-characterized FasL (CD95L)-Fas (CD95) DR ligand-receptor pair, additional molecules including TWEAK (DR3 ligand) and TRAIL (DR4,5 ligand) have recently been identified as capable of activating the caspase system and subsequent apoptosis [101–104] (Fig. 3). Although the physiologic functions of DR3, 4, and 5 are not presently known, the expression of TRAIL and TWEAK on T cells may be important contributors during transplant responses to this process.

During the past several years, a number of experimental allogeneic BMT studies have used donor grafts that are unable to mediate either perforin/granzyme or FasL-Fas dependent killing [105–110]. Transplantation of perforin-deficient T cells results in a marked delay in the onset of GVHD-associated weight loss and mortality in both MHC and miH incompatible systems [105,106]. However, these studies also revealed that although greater numbers of perforin-deficient T cells were required to induce GVHD with kinetics comparable to that caused by normal T cells, weight loss and mortality were, in fact, induced in the absence of perforin-dependent killing. Moreover, the clinical signs of GVHD, including kyphosis, alopecia, skin lesions, and diarrhea, as well as histopathologic changes in the skin, liver, and lymphohematopoietic compartment, were all eventually observed [105,106].

Studies employing donor T-cell subsets show that granzyme B–deficient $CD8^+$ T cells induced significantly less mortality than wild-type T cells in experimental transplants across a single MHC I mismatch or complete MHC I and II mismatches [107,110,111]. Titration of donor T cells demonstrated a three- to fivefold difference in the number of perforin/granzyme B–deficient $CD8^+$ T cells required to mediate comparable GVHD, but titration of perforin/granzyme B–deficient $CD4^+$ T cells produced conflicting results [107,110,111], and thus the contribution of this pathway to GVHD induced by $CD4^+$ T cells is less certain.

Although the perforin/granzyme pathway may contribute to tissue injury, it is now clear that it is not necessary to generate tissue damage. Because increased numbers of perforin deficient cells can induce comparable weight loss and mortality with the same kinetics as normal donor cells, the killing of host cells via perforin may be a contributor to the "afferent" events (phases 1 and 2) of acute GVHD. Studies have shown that macrophages and dendritic cells (APC populations) can be killed by both perforin- and FasL-dependent pathways [112–114]. The amplification/expansion of donor T cells may be facilitated by efficient lysis of host cells and the subsequent release of host alloantigens and cytokines from host APCs. Early during GVHD, perforin/granzyme function directed at host cells may also protect alloreactive donor T cells by eliminating host T/NK-populations that can regulate or impede donor T cells [115].

Perforin-deficient T cells retain the capacity to mediate FasL-dependent killing. Accordingly, experiments have been performed to examine the consequences of transplanting donor cells unable to cause Fas-mediated apoptosis. These studies have used T cells from mice with a naturally occurring genetic mutation (*gld/gld*) resulting in a FasL protein that cannot trimerize Fas and therefore fails to induce Fas signaling [116]. Transplantation of donor T cells with functionally defective FasL in $CD8^+$-dependent models resulted in only modest delay in weight loss and in a small increase in median survival time (MST) [106,109]. However, the ability to induce acute GVHD by FasL-defective donor cells was significantly impaired in $CD4^+$-dependent models [107,109,110]. These studies suggest that FasL is more important for GVHD induced by $CD4^+$ T cells whereas perforin/granzyme is more important for GVHD dependent on $CD8^+$ T cells [106–110].

These murine models also suggest that perforin and FasL pathways are not equally important for all target organs. For example, FasL-mediated cytotoxicity is an important effector pathway in hepatic GVHD, even in $CD8^+$ T-cell–dependent models [106]. A number of non-GVHD studies have reported that the liver appears particularly sensitive to Fas-induced injury [117–120]. Consistent with these findings, a recent study observed that transplantation of MiH-disparate allogeneic T cells failed to induce hepatic GVHD in Fas-deficient recipients (M. Van den Brink, personal communication). Another recent study reported that administration of anti-FasL (but not anti-TNF antibody) significantly blocked the hepatic damage occurring in an MHC nonidentical GVHD model [121].

Cutaneous GVHD may also be mediated by FasL cytotoxicity, even in $CD8^+$ T-cell–dependent GVHD systems [106]. Minimal inflammation was observed in skin sections from recipients of miH-mismatched FasL-defective donor T cells, suggesting that FasL-Fas was an effector pathway of GVHD in the skin. Although constitutive Fas expression in the skin is low, keratinocytes have been found to express Fas during viral infections and certain cutaneous diseases [122]. Cytokines induced during GVHD may also upregulate Fas expression in the skin, and several studies have demonstrated that anti-TNF-α antibody inhibited the development of skin GVHD [78,123]. TNF-α and INF-γ can upregulate FasL expression on keratinocytes [124], and a recent investigation found that the introduction of either anti-FasL or anti-TNF antibody diminished GVHD skin lesions [121]. Injection of both antibodies completely prevented skin damage, and thus signaling through both Fas and TNF receptors may both contribute to GVHD-associated skin injury.

In contrast to FasL involvement in hepatic and cutaneous GVHD, TNF-α plays a dominant role in intestinal GVHD damage. Anti–TNF-α but not anti-Fas antibody blocked gastrointestinal damage in MHC-mismatched GVHD [121]. FasL may play a small role, however, because one study reported that intestinal intra-epithelial lymphocytes from a P→F1 GVHD model exhibited increased FasL-mediated intestinal apoptosis after transfer to normal mice

[125]. Additional studies are needed to further elucidate the involvement of these molecules in intestinal GVHD.

Finally, transplant of FasL-defective donor T cells has also resulted in a diminution in the level of lymphoid (splenocyte and thymocyte) depletion in recipients [106, 108,109]. Similar to results detected in the skin, treatment with either anti-FasL or anti–TNF-α antibody reduced lymphoid depletion [121].

The use of a perforin/granzyme and FasL cytotoxic double-deficient (cdd) inoculum provides the opportunity to address whether other effector pathways are capable of inducing GVHD target organ pathology. Braun *et al.* [126] reported that perforin/FasL-defective donor spleen cells were unable to induce GVHD lethality in recipients transplanted after 6.5 cGy TBI with cells from MHC I/II–mismatched donors. However, resistance by the host after nonlethal TBI may have significantly diminished the GVHD capacity of the donor inoculum. Two subsequent studies transplanted cdd donor cells into MHC-mismatched recipients conditioned with lethal TBI and observed significant GVHD [127,128]. T cells concomitantly unable to mediate both perforin/granzyme- and FasL-dependent cytotoxicity were capable of inducing GVHD, although considerably greater numbers of cdd cells were required. A recent study employing granzyme B- and FasL-deficient T-cell subsets suggests that such $CD8^+$ T cells cannot induce GVHD [110], whereas $CD4^+$ T cells unable to mediate perforin- and FasL-dependent cytotoxicity can (Z. Jiang, R. Levy, unpublished observations). These results demonstrate that other effector molecules can cause severe GVHD in the absence of perforin/granzyme and FasL. Future studies will undoubtedly focus on the newly emerging death receptor ligands, together with cytokines including TNF-α.

In summary, recent investigations have begun to define the contributions of cell-mediated cytotoxicity via both perforin/granzyme- and FasL-dependent pathways to both systemic GVHD and to GVHD target organ damage. The newly emerging molecular pathways of death signals should provide more complete and precise definitions of requirements for GVHD-induced pathogenesis. Since both $CD4^+$ and $CD8^+$ cells mediate GVHD and graft-vs.-leukemia (GVL) activity, assessing the relative contributions of each of the cytotoxic pathways in individual subsets may help in the potential disassociation of GVHD from GVL. As our understanding deepens of the relative contribution of each of these pathways to GVHD pathology in individual GVHD target organs, novel strategies may emerge to optimize prophylaxis and therapy for individual host tissues.

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